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WHITE PAPER No. 19

Controlled Cultivation of Stem Cells – Factors to Consider When Thinking of Scale-Up

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Executive Summary

Great hopes and expectations are linked to stem cells as a tool for drug discovery and to stem cell-derived products in therapeutic applications. Though several products have made it to commercial stage, most of the research is still performed in small scales using simple cultivation systems such as spinner flasks or T-flasks.
Drug approval procedures however require a detailed knowledge of the process, reproducible results and a comprehensive documentation. At this point, the mentioned cultivation systems quickly reach their limits. To achieve production level yields these simple systems have limited economies of scale.
Controlled bioreactors, widely established in traditional cell culture applications, can be the key to establish and optimize reproducible cultivation processes. Adapted to the special requirements of sensitive stem cells they provide a powerful tool for scale-up.

Introduction

Stem cells and their (potential) applications in drug screening, toxicology testing and regenerative medicine are not only on everyone's lips, but several hundred stem cell-derived products have made it to the clinic since Geron®'s GRNOPC1 was approved for Phase I in 2009 [1]. Though in the following the industry suffered some setbacks like Geron's exit from the stem cell field and the massive fall of Dendreon®'s stocks in 2011, over 100 stem cell-derived products are commercially available [2].
The production of high quality cells in sufficient amounts remains one of the challenges that have to be addressed while processes need to be documented extensively to meet regulatory demands. Cultivation systems such as T-flasks and spinner/shaker flasks are widely used in stem cell research providing viable and simple methods for cultivation. However, they are limited in terms of control and scalability (reviewed in [3, 4]):

> Key cultivation parameters such as oxygen tension and pH are not monitored or even controlled in real-time which makes it difficult to establish a robust and reliable process.
> Medium perfusion and direct monitoring of cell viability and metabolic activity cannot easily be handled.

> T-flasks and spinner flasks require a lot of space when larger volumes are needed. They offer few economies of scale therefore being difficult and expensive to scale up.
> Documentation and analysis is labor-intensive and time-consuming with limited potential for automation. Thus, regulatory procedures, quality assurance and quality control need to be simplified.

Scalable bioreactor design and minimum working volumes

At research and process optimization stage, small working volumes help saving valuable cell material and resources. Since stem cell research is associated with great expectations for future applications in diagnostics and therapeutics, cultivations at the same time need to be reproducible and scalable in order to gain sufficient yields of cell material for further applications [5].
Stirred-tank bioreactors of all sizes have been used in cell culture for many years. Compared to uncontrolled systems such as shake flasks they provide extensive options for monitoring and control of key cultivation parameters in real-time. Besides the vessel design itself (aspect ratio), stirring, feeding, monitoring and sampling mechanisms and control factors need to be constructed in a way that directy supports scale-up. In that way, these bioreactors help to establish optimum growth conditions that can be reproduced and transferred to larger working volumes. Industry standard design of bioreactors used in research and early process development facilitates scale-up and ensures consistency throughout development phases.

Smooth agitation minimizing shear forces

In dynamic cell culture mixing is essential for achieving and maintaining a controlled environment. Apart from the impact on differentiation, the resulting shear stress might harm sensitive stem cells. In consequence, agitation should be high enough to keep cell aggregates or microcarriers in homogenous suspension, but has to be lower than the critical level damaging cells or aggregates. The chosen bioreactor system should therefore be able to provide low stirring speeds. Mixing studies can help evaluating the optimum agitation set-up when establishing a process [6, 7].
Besides the stirring speed, impeller design has a major influence on mixing and cell aggregate formation. In studies both designs, pitched blade impellers (widely used in traditional cell culture applications) as well as adapted

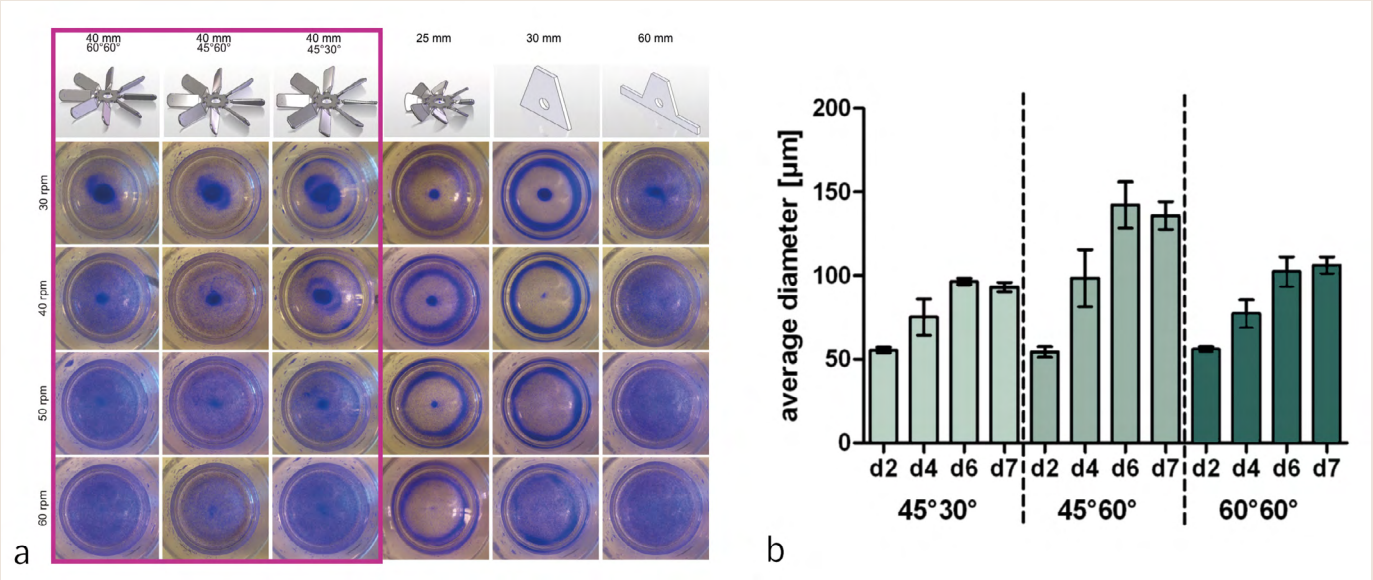

Figure 1: Impact of impeller design and stirring speed on mixing capabilities (left) and cell aggregate formation (right) in stirred suspensions using flat-bottom glass vessels (Ø 70 mm).
a Stained microcarriers were stirred in 100 mL buffer for 10 min at different speeds. **b** Aggregate sizes of human induced pluripotent stem cells using three different pitched blade impellers were measured at day 2, 4, 6, and 7 of the culture in 2 – 3 independent runs each. [7]

paddle-type agitators, have been proven effective even at low speeds (fig. 1) [7]. However, when setting up a new process individual agitation conditions need to be tested and evaluated to achieve optimum cell growth.

Tight control of oxygen tension

Depending on the tissue type that the stem cells originate from, hypoxic conditions will be favored over the atmospheric („normoxic") oxygen concentration of 21 % [reviewed in 10]. For example in bone marrow oxygen concentration is below 4 % pO_2, equating to approximately 19 % dissolved oxygen (DO). The impact of oxygen on growth and differentiation has been investigated for various types of stem cells such as induced pluripotent [8] and embryonic stem cells (ESCs) [9]. Studies report that both proliferation and differentiation of investigated cells strongly depended on the oxygen tension during cultivation. For example, the formation of blood or endothelial cells from mouse ESCs was shown to be opposed depending on the oxygen concentration: Whereas a hypoxic environment stimulated differentiation towards hematopoietic progenitor cells, the path towards endothelial cell development was suppressed at low oxygen tensions (fig. 2).
Stable growth conditions during experiments are hence prerequisite for optimizing cultivation, for example to achieve higher cell growth and suppress differentiation. Mimicking the physiological O_2 level, advanced stirred-tank bioreactor systems can help on the way to a better understanding of the cellular metabolism. They enable researchers to establish an environment with steadily controlled oxygen tension even at hypoxic levels. Measuring the current DO concentration in the medium automated feedback control loops can be used to ensure a predefined oxygen environment within the culture. Cascaded gassing strategies, for example by first enriching from air to pure oxygen and then shifting to a slight gas flow adjustment, can be defined by the user to meet individual needs of the culture.

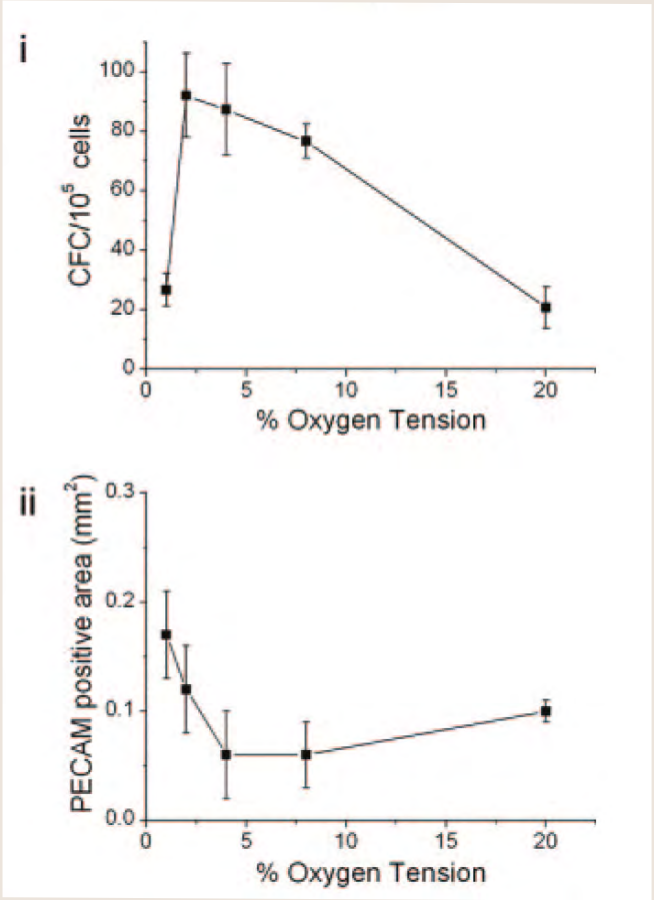

Figure 2: Differentiation pathways of mouse embryonic stem cells depending on oxygen tension (1, 2, 4, 8 and 20 %). Cells were cultivated in stirred-tank bioreactors (DASGIP® Parallel Bioreactor System) forming embryoid bodies, and harvested and analyzed after 7 days.
i Formation of hematopoietic progenitor cells was determined via their morphology in an ME-CFC (myleloid-erythroid colony forming cell) assay. **ii** Endothelial cell development was detected by staining with platelet-endothelial cell adhesion molecule-1 (PECAM). [9]

Precise pH control

The activity of cell metabolism-controlling enzymes is strongly pH-dependent, and thus the resulting growth and differentiation of stem cells is affected as well: Suboptimal pH conditions will lead to inhibition or altered differentiation. In contrast to the simple addition of buffers and a predefined CO_2 surrounding, resulting in pH shifts and inhomogeneities during cultivation, stirred-tank bioreactor systems allow accurate control of pH levels. pH values are measured using industry standard pH sensors and precisely regulated by adjusting CO_2 gassing or addition of acid and base.

External analyzer integration

The integration of sampling and analytical laboratory devices is suitable for automation of processes and opens up further possibilities to closely monitor the cultivation.

Online monitoring of cell viability and metabolism is enabled with a bidirectional communication between the devices via standard protocols (OPC) allowing for sampling on demand or other process-triggered feedback control loops. For example, nutrient analyzers such as the Cedex® Bio (Roche Diagnostics®, Mannheim, Germany) can be integrated via OPC. This permits online monitoring and direct conclusions on the cell growth. Automated feedback loops can be set up. Seamlessly integrating external devices with the bioreactor system thereby supports researchers and process engineers in the stem cells field to establish integrated processes and automation.

Extensive documentation and data analysis

A successful regulatory strategy requires comprehensive documentation early in development of new products. Advanced bioreactor systems should include state-of-the-art control software and information management, enabling all process data to be stored in a central database. This should not be limited to the extensive online data generated throughout the process, but also apply to offline data from post-expansion quality control where stem cell marker assays and differentiation assays (e.g. immunoassays, PCR-based methods) are conducted to evaluate the quality of the stem cell population [11]. Integrated recipe management, standard operating procedures (SOP) and managed access ensure a high level of safety and will minimize error sources. When it comes to data analysis easy-to-use queries, real-time retrieval of key process information and batch-to-batch comparison of data and trends ease process engineers' daily work while leading to a solid process understanding.

Single-use technology

Facilitating qualification and validation single-use systems call more and more attention in pharmaceutical cell culture. They are usually easy to use, reduce the risk of cross-contaminations and result in labor and capital investment savings as well as reduced turn-around times. Rigid-wall single-use solutions mimicking traditional stirred-tank designs comprise the advantages of glass and stainless steel vessels with regards to reproducibility and scale-up. Studies have proven these bioreactors to be suitable for scalable expansion of stem cells being cultivated on microcarriers or as cell-only aggregates [12, 6]. Clinical doses were achieved in a single run using a benchtop scale single-use bioreactor. Monolayer virgin plastics produced without the use of softeners simplify material validation and eliminate concerns regarding leachables and extractables. Premium suppliers use USP class VI-certified materials.

Summary

Controlled bioreactor systems have the potential to streamline stem cell research and process development by establishing processes including real-time monitoring of key cultivation parameters, feedback control and automation. Comprehensive documentation, advanced software tools and statistical methods pave the way to an integrated process development taking regulatory and quality aspects into account even in early stages. Scalable bioreactor design helps gaining reproducible results and sufficient cell numbers for a clinical approach while single-use technologies reduce labor time and simplify validation.

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4-fold system for single-use vessels	76DX04CCSU
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